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HEAT SHOCK PROTEINS IN CULTURED GILL CELLS OF COLOR CARP *CYPRINUS CARPIO* L.

CHEN-CHUN KU and SHIU-NAN CHEN

Department of Zoology, National Taiwan University, Taipei, Taiwan 10764, Republic of China

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Chen-Chun Ku and Shiu-Nan Chen (1991) Heat shock proteins in cultured gill cells of color carp *Cyprinus carpio* L. *Bull. Inst. Zool., Academia Sinica* **30**(4): 319-330. The exposure of cultured gill cells of color carp (CCG) to an elevated temperature resulted in the over-production of four heat shock proteins: *hsp*87, *hsp*70, *hsp*33 and *hsp*27. The kinetics of their syntheses at 37°, 40° and 43°C were studied. With various heat shock treatments, the synthesis of *hsp*70 was the most rapid and prominent, and *hsp*27 was the slowest. Prolonged heat shock at 41°C beyond 6 h resulted in decreased syntheses of *hsp*87 and *hsp*70, and the synthesis of *hsp*33 ceased, but the synthesis of *hsp*27 continued. However, upon continued heat shock at 37°C only *hsp*87 and *hsp*70 were synthesized.

An immunofluorescent stain showed that *hsp*70 was mainly located in the nuclei of the CCG cells, and *hsp*33 and *hsp*27 were located in the cytoplasm with an intense perinuclear granules after heat shock at 40°C for 10 h. When the cells were recovered at growth temperature for 24 h, *hsp*70 disappeared, but the perinuclear granules of *hsp*33 and *hsp*27 could still be detected in the CCG cells. Prior-treatment of CCG cells with low doses (3 μg/ml) of actinomycin D for 1 h inhibited the induction of heat shock proteins, which indicates that the control of the expression of heat shock proteins is regulated at the transcriptional level. All of the four heat shock proteins were degraded during electrophoresis.

Key words: Heat shock proteins, Fish cells, Arsenite, Cadmium.

Heat shock proteins (*hsps*) are produced when a cell is stressed by various enviromental insults. These insult factors include temperature and a variety of chemical compounds such as sulfhydryl reagents, various heavy metals, iodoacetamide, sulfhydryl, ethanol and other aliphatic alcohols (Lee and Hahn, 1988). However, these factors are not equally effective among all species (Heikkila *et al.*, 1982; Kothary and Candido, 1982; Misra *et al.*, 1989; Schlesinger, 1986). Moreover, the response has now been shown to occur in many different systems, ranging from bacteria (Lindquist, 1986; Yamamori and Yura, 1980), protozoas (Craig, 1985), yeasts (McAlister and Finkelistein, 1980), sea urchins (Giudic, 1989), insects (Berger and Woodward, 1986), parasites (Newport *et al.*, 1988), plants (Nover *et al.*, 1983), up to all higher mammalian tissues (Currie and White, 1982) and a number of eukaryotic cell lines (Craig, 1985).

Little is known about the function of *hsps*, but they appear to be involved in the development of thermotolerance and may be part of a general protective mechanism for cells under physiological

and environmental stresses (Lindquist, 1986; Schlesinger, 1986). *Hsp*s are also believed to be among the first products resulting from zygotic gene activity in early mouse embryos, and it is suggested that they may play an important role in normal development and differentiation (Bensaude *et al.*, 1983; Kurtz *et al.*, 1986). Thus, it will be important to compare the results of several organisms to determine if these different proteins function identically or if differences in function have evolved as the species diverged.

Studies of heat shock responses in fish cell lines were previously performed mainly on cultured RTG-2 and RTH cells derived from rainbow trout, *Salmon gairdnerii* (Kothary and Candido, 1982; Kothary *et al.*, 1984; Mosser *et al.*, 1986; Misra *et al.*, 1989), CHSE-214 cells derived from chinook salmon embryo cells (Heikkila *et al.*, 1982; Gedamu *et al.*, 1983) or TO-2 cells derived from the ovary of adult tilapia hybrid of *T. mossambica* and *T. nilotica* (Chen *et al.*, 1988). The responses of fish cells to heat, sodium arsenite or metal ions are very similar to those found in *Drosophila* (Lewis *et al.*, 1975). At the molecular level, the cDNA of trout *hsp*70 has been cloned and sequenced, and shows extensive homology in the *hsp*70 genes of both *Drosophila* and yeast (Kothary *et al.*, 1984b).

This paper attempts to describe the dynamic state of *hsp*s in the cultured gill cell line of color carp, *Cyprinus carpio* (CCG), after heat shock or sodium arsenite treatment. Polyclonal antibodies were used to detect the localization of the *hsp*s before and after the heat shock.

MATERIALS AND METHODS

Cells and culture conditions

The CCG cell line originated from the gill of color carp fry. The cells were maintained in Leibovitz's L_{15} medium (Hazleton) supplemented with 10% fetal calf serum (Sera-lab Ltd., England), 100 U/ml penicillin-streptomycin and 2.5 μg/ml amphotericin B (Boehringer Mannheim GmbH, West Germany). The cultures were kept in an incubator at 31°C. The pH value of the culture media was maintained at approximately 7.0 during culturing and heating.

Induction of heat shock proteins by heat

The heating of monolayers of cells was carried out in precalibrated water baths. Monolayers of cells in parafilm-sealed petri dishes or flasks were submerged under water for acute heat shock treatments. The water-bath temperature was controlled to within +/− 0.1°C. For long-term thermal exposure (37°C and 40°C), the cultures were shifted to precalibrated incubators. The temperatures inside the incubators were controlled to within +/− 0.2°C.

Induction of stress proteins by arsenite and cadmium

Confluent monolayers of cells were exposed to sodium arsenite or cadmium chloride from 0 to 150 μM for 1 h at 31°C.

Protein labelling

Cells were labelled in methionine-free medium with 20 μCi/ml ^{35}S-methionine (specific activity 1,209.3 Ci/mmol) for 2 h immediately after various treatments.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze protein patterns following the procedures of Laemmli (1970). Cells were rinsed with cold phosphate-buffered saline (PBS) and lysed in an equal volume of SDS-PAGE sample buffer (0.07 M Tris, pH 6.8, 11.2% glycerol, 3% SDS, 0.01% bromophenol blue and 5%

β-mercaptoethanol). The lysates were boiled for 5 min and analyzed on a 10% polyacrylamide gel with 5% polyacrylamide stacking gel in the buffer system of 0.025 M Tris (pH 8.8), 0.192 M glycine, 0.1% SDS and 0.002 M EDTA. After electrophoresis, the gels were fixed with 30% methanol/10% acetic acid for 1 h. The gels were then dried and exposed to Duput cronex X-ray film at −70°C for 6 days. Autoradiograms were developed in a Dupont cronex X-ray developer. In order to compare the intensities of protein bands, equal volumes from each sample were loaded into the slots of the gels. The X-ray films were scanned with a computerized scanning densitometer (GS300; Hoefer).

To rerun the gel blocks and gel stripes, the sample gels were directly incorporated into 5% stacking gel, then embedded with 5% stacking gel. In all experiments, 10% SDS-PAGE was used.

Indirect immunofluorescent stain

About 10^5 cells were plated directly onto a 35×10 mm petri dish (Nunc., Denmark) and allowed to adhere for at least 24 h. The cells were fixed in 3.7% paraformaldehyde in PBS for 10 min, followed by immersion in 0.2% Triton X-100 in PBS for 2 min and a PBS wash. Rabbit anti-*hsp*72 (prepared from color carp testis cell line, CCT), rabbit anti-*hsp*33 or *hsp*27 (prepared from color carp gill cell line, CCG) antibody was diluted 1:500 in PBS and incubated with cells for 60 min at room temperature. The cells were rinsed with PBS and incubated for 30 min with a 1:20 dilution of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (Jackson Immunoresearch). The dishes were then rinsed, mounted in phosphate-buffered glycerol, and observed with a microscope equipped with epifluorescence optics. Samples were photographed with Kodak 35-mm Tri-X film shot at 400 ASA for 50 or 60 sec.

RESULTS

Cell viability and proliferation at different temperatures

The ability of CCG cells to persist for 6 days at temperatures of 18°, 24°, 31°, 37° and 40°C has been previously reported (Ku *et al.*, 1911); monolayers endure at all temperatures. However when cultured at 43°C for 30 min, very few to no cells were attached to the growth surface, and many floating cell fragments were observed in the culture medium. At 40°C, proliferation gradually subsided; cells lost their normal shape and became more flattened. Optimal cell growth occurred at both 37° and 31°C. At 18°C, cells remained viable but grew slowly.

CCG cells grew well when treated with sodium arsenite or cadmium chloride of less than 150 μM for 1 h.

Kinetics of *hsps* synthesis after heat shock at 43° or 40°C

Monolayers of CCG cells were heated to 43° or 40°C for 3, 5, 10, 15, 30 and 60 min. After being heated, cells were labelled with ^{35}S-methionine at 31°C for 2 h in a methionine-free medium. The proteins synthesized during 2 h after heat shock were analyzed by one-dimensional gel electrophoresis. The gel patterns (Figs. 1 and 2) clearly demonstrated that four heat shock proteins were identified; these were *hsp*87, *hsp*70, *hsp*33 and *hsp*27.

*Hsp*70 was the major synthesized protein in CCG cells after various periods of heat shock at 43°C (Fig. 1). Its synthesis was enhanced after 3 min and reached its maximal rate after 10 min at 43°C. However, after 15 min at 43°C, the synthesis of all other proteins except *hsp*70 ceased. Synthesis of *hsp*87 was enhanced and reached its maximal rate after 3 min, and that of *hsp*33 was after 10 min of

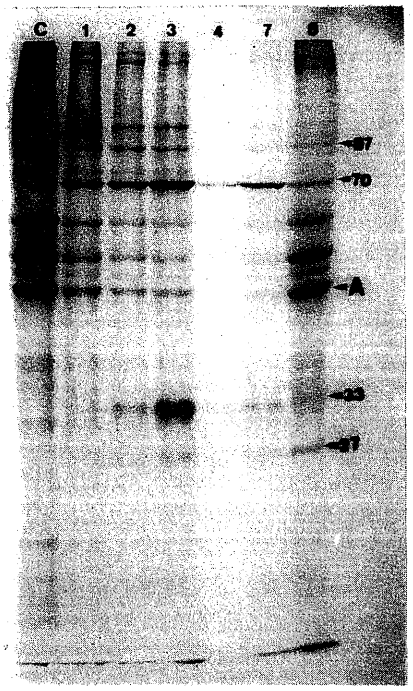


Fig. 1. Autoradiogram showing the enhanced synthesis of *hsps* after acute heating at 43°C. CCG cells were heated at 43°C for (C) 0, (1) 3, (2) 5, (3) 10, (4) 15 min, then labelled for 2 h at 31°C. After being heated at 43°C for 10 min, the cells were labelled with ^{35}S-methionine during recovery periods of (7) 4-6, or (8) 8-10 h of recovery at 31°C.

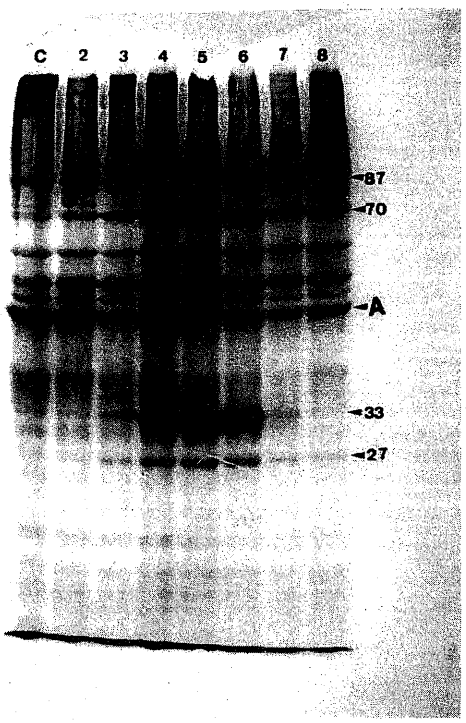


Fig. 2. Autoradiogram showing the enhanced synthesis of *hsps* after chronic heating at 40°C. CCG cells were heated at 40°C for (C) 0, (2) 5, (3) 10, (4) 15, (5) 30, (6) 60 min, then labelled for 2 h at 31°C. After being heated at 40°C for 30 min, the cells were labelled with ^{35}S-methionine during recovery periods of (7) 4-6, or (8) 8-10 h at 31°C.

treatment at 43°C. Less *hsp27* was synthesized.

Fig. 2 shows the kinetics of *hsps* syntheses after various periods of heat shock at 40°C. The four major *hsp*s were enhanced by all of these treatments (5, 10, 15, 30 and 60 min at 40°C). In this set of treatments, *hsp87* was enhanced after 10 min and reached its maximal rate after 15 min; *hsp70* was enhanced after 5 min and reached its maximal rate after 10 min; *hsp33* was enhanced after 15 min and reached its maximal rate after 60 min. The synthesis of *hsp27* exhibited a similar temporal pattern to that of *hsp33* but less *hsp27* was generated.

Kinetics of *hsps* synthesis during heat shock at 40°C

Cells were heated continuously at 40°C and labelled from 4 to 6, 8 to 10, 36 to 38, 60 to 62, 84 to 86, and 117 to 119 h. The proteins were analyzed by one dimensional gel electrophoresis (Fig. 3). Syntheses of *hsp87*, *hsp70* and *hsp27* were continuously enhanced during the experimental period; however, *hsp33* ceased being synthesized after 7 h at 40°C. Both *hsp87* and *hsp70* reached their maximal synthesis rates during 4-6 h and declined after 8 h. *Hsp33* was detected exclusively before 6 h and returned to control levels

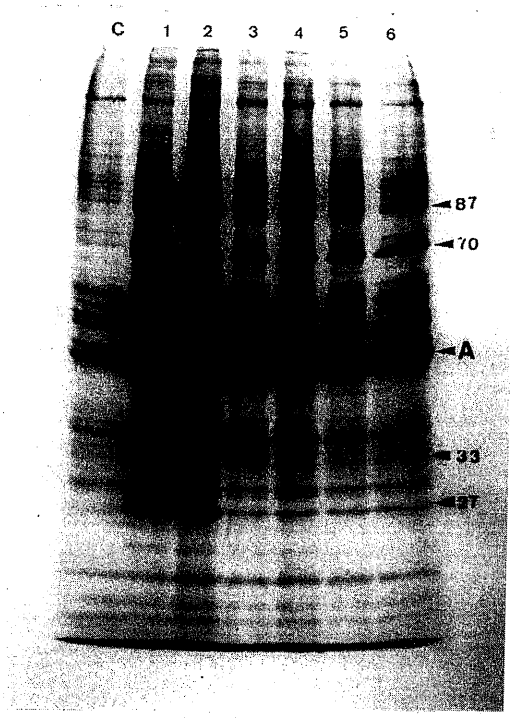


Fig. 3. Autoradiogram showing the enhanced synthesis of *hsps* during chronic heating at 40°C. CCG cells were heated continuously at 40°C and labelled (C) control, (1) 4 to 6, (2) 8 to 10, (3) 36 to 38, (4) 60 to 62, (5) 84 to 86, (6) 117 to 119 h.

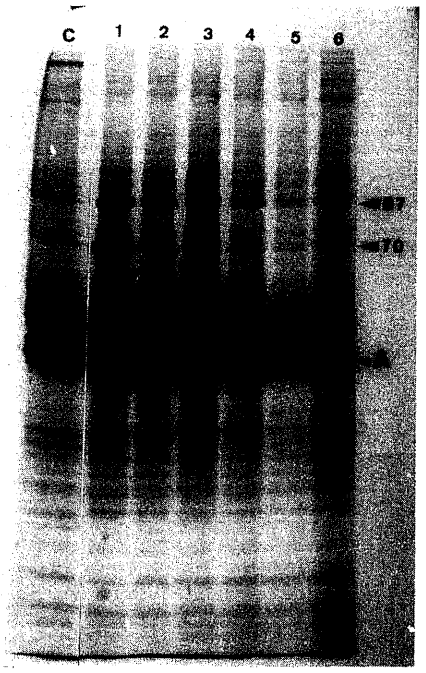


Fig. 4. Autoradiogram showing the enhanced syntheses of *hsps* during thermal adaptation heating at 37°C. CCG cells were heated continuously at 37°C and labelled (C) control, (1) 4 to 6, (2) 8 to 10, (3) 36 to 38, (4) 60 to 62, (5) 84 to 86, (6) 117 to 119 h.

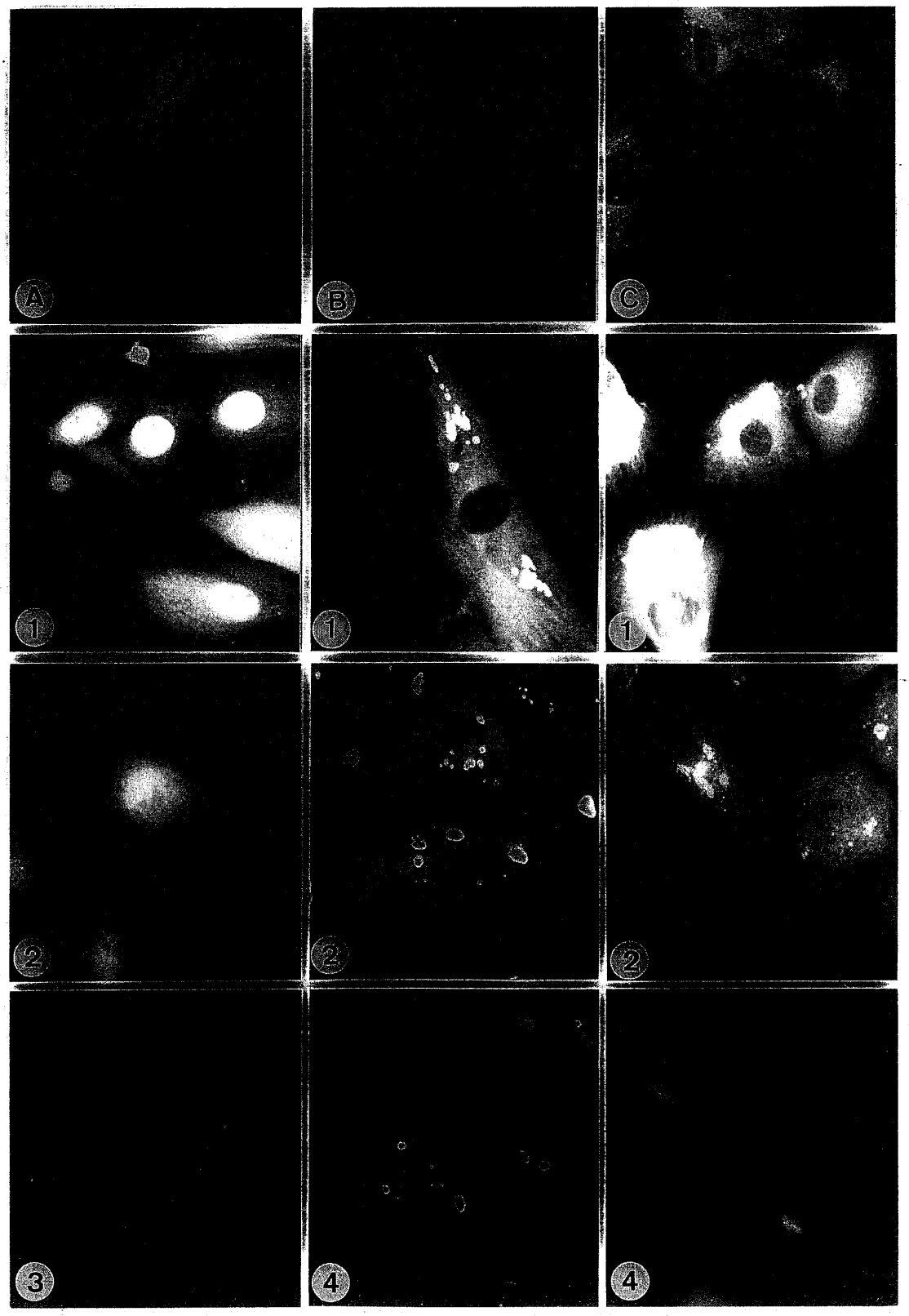
by 8 h. *Hsp27* reached its maximal synthesis rate during 4–6 h at 40°C.

Kinetics of *hsps* synthesis during heat shock at 37°C

When CCG cells were incubated at 37°C, cells proliferated normally. The proteins synthesized during thermal adaptation were analyzed by labelling cells 4 to 6, 8 to 10, 36 to 38, 60 to 62, 84 to 86 and 117 to 119 h after transferring cultures from 31° to 37°C. The results are shown in Fig. 4. *Hsp87* and *hsp70*, which were synthesized at 40°C, also appeared in cells heated to 37°C but in lesser quantities. *Hsp33* and *hsp27* were not observed. Syntheses of both *hsp87* and *hsp70* reached maximal rates during 4–6 h and declined after 8 h.

Duration of *hsps* after heat shock

Monolayers of CCG cells were heated at 43°C for 10 min (Fig. 1, lane 7 and 8) or 40°C for 30 min (Fig. 2, lane 7 and 8) then incubated at 31°C. While incubated at 31°C, cells were labelled with ^{35}S-methionine during 4–6 or 8–10 h in a methionine free medium. The results show that after heat shock at 43°C for 10 min, the syntheses of many normal proteins were suppressed during a 4–6 h period of recovery. The overall protein syntheses of the heated cells returned to the normal state during recovery periods of 4–6 h after 30 min exposures at 40°C and 8–10 h after 10 min exposures at 43°C.


A
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Localization of *hsps* before and after heat shock

An immunofluorescence assay with polyclonal antibodies specific to *hsp*s was used to detect the localization of *hsp*s before and after heat shock. CCG cells were heated at 40°C for 10 h then transferred to 31°C. After recovery for 0, 14, 24 and 36 h, the cells were reacted with rabbit antibodies: anti-*hsps*70, anti-*hsps*33 or anti-*hsp*27. The immunofluorescent stains showed that *hsp*70 was absent from CCG cells grown at 31°C (Fig. 5A). Following heat shock at 40°C for 10 h, intense nuclear staining with a slight increase in the cytoplasmic staining appeared in all cells. By 14 h after treatment the cells were returned to 31°C and the intensity of staining of *hsp*70 had decreased substantially. In addition the stain appeared to diffuse into the cytoplasm. By 24 h after treatment the intensity of staining was at the preheat shock level.

*Hsp*33 was very weak in unstressed CCG cells (Fig. 5B). Following heat shock treatment at 40°C for 10 h, an intense cytoplasmic staining with prominent granules was observed. After a 14 h recovery period at 31°C, the intensity of cytoplasmic staining had dramatically decreased but the intensively stained cytoplasmic aggregates could still be observed. After recovery for 36 h, the cytoplasmic stain had disappeared, and only the cytoplasmic aggregates were apparent.

*Hsp*27 could be detected in the nucleus of about half of the tested unstressed CCG cells; the other cells had very diffused staining in the cytoplasm (Fig. 5C). However, after heat shock at 40°C for 10 h, only bright cytoplasmic staining was observed. When cells were allowed to recover at 31°C for 14 h after the heat stress, the intensity of cytoplasmic staining diminished, but prominent cytoplasmic granules continued to be prominent. After a 36 h period of recovery, very few cells with cytoplasmic granules could be detected among the tested cells.

Self-degradation of heat shock proteins in CCG cells

Identical aliquots of heat-shocked CCG cell extracts were electrophoresed in one-dimensional SDS-PAGE and then cut out from the wet gel as shown in Fig. 6A. Gel blocks (*hsp*87, 70, 33 and 27) from one lane were inserted into a new gel and rerun to give the pattern in Fig. 6B. The results indicate that each *hsp* had characteristic and relatively stable breakdown products. For example, *hsp*70, the most apparent, was mainly reduced to 43, 27 kilodalton and other minor degradative products in the lane. The same results were observed by cutting the whole strip rather than a block after one-dimensional electrophoresis. In this case, the second run was displayed as a diagonal and a vertical pattern (Figs. 6C and D). This indicates that all of these *hsp*s have a self-degradation ability. In 1985, Mitchell *et al.* also reported that *hsp*70 in *Drosophila*, CHO and a mouse cell line degraded spontaneously. These results indicated that all *hsp*s in CCG cells created slow proteolytic action upon themselves.

Fig. 5. Duration of *hsps* after chronic heating at 40°C. Cells were held at 31°C (A, B, or C) then heated at 40°C for (1) 10 h and allowed to recover at 31°C for (2) 14, (3) 24, (4) 36, respectively. Cells were fixed with 3% paraformaldehyde, permeabilized with 0.2% Triton X-100, and stained with rabbit anti-CCT *hsp*72 antibodies (column A), anti-CCG *hsp*33 antibodies (column B), or anti-CCG *hsp*27 antibodies (column C) followed by FITC-conjugated anti-rabbit IgG antibodies. ×370.

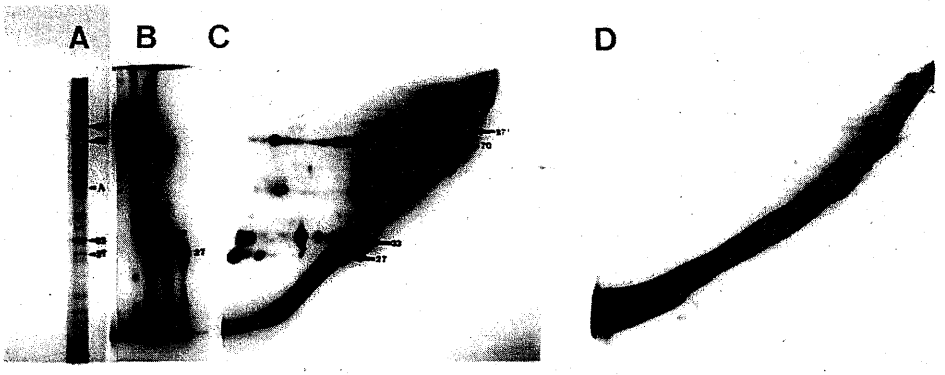

Fig. 6. Degradation of *hsps* in CCG cells during electrophoresis. (A) Heat-shocked cells. (B) A rerun of blocks containing *hsp*87, 70, 33 and 27. (C) A rerun of gel strip from heat-shocked cells and (D) control cell.

Effect of actinomycin D in the induction of *hsps*

Pretreatment of CCG cells with low doses (3 μg/ml) of the transcriptional inhibitor actinomycin D for 1 h inhibited the induction of heat shock proteins. However, treatment with actinomycin D after heat shock with the same doses and duration as the pretreatment did not inhibit *hsp* induction (Fig. 7), suggesting that control of the expression of *hsp*s was regulated at the transcriptional level.

Induction of stress proteins by arsenite and cadmium

In order to investigate the syntheses of stress proteins in fish cell lines, CCG cells were exposed to various concentrations of arsenite and cadmium for 1 h. After they were washed, cells were labelled for 2 h at 31°C in a methionine free medium. The proteins synthesized during a 2 h period after stress were analyzed by one dimensional SDS-PAGE. The gel patterns (Fig. 8) clearly demonstrated that only arsenite-enhanced *hsp*70 synthesis occurred in the CCG cells. It began to be synthesized at 25 μM and reached its maximal rate at 100 μM after 1 h of treatment. This result was very similar to that reported for *Saccharomyces cerevisiae* in which it was found that arsenite enhances synthesis of only *hsp*74

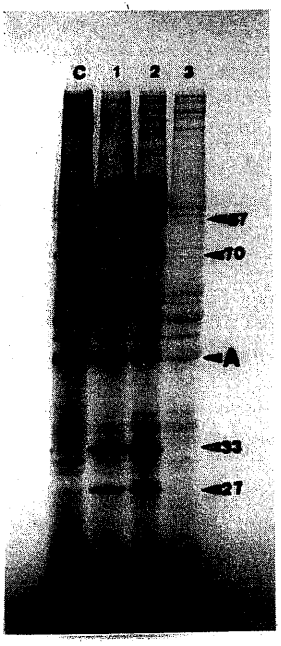

Fig. 7. Effect of actinomycin D in the induction of *hsps*. (C) CCG cells at 31°C, (1) cells were heated at 40°C for 1 h. (2) treatment with 3 μg/ml actinomycin D after heat shock for 1 h. (3) pretreatment of CCG cells with 3 μg/ml actinomycin D for 1 h, then heated at 40°C for 1 h. After each treatment, cells were washed and labelled with 20 μCi/ml ^{35}S-methionine for 2 h.

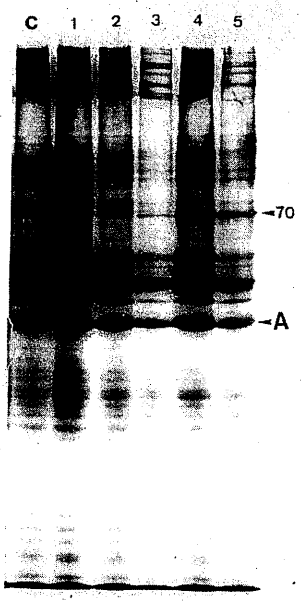

Fig. 8. Induction of stress proteins by arsenite. CCG cells are exposed to various concentrations of arsenite for 1 h. After being washed, cells are labelled for 2 h at 31°C in a methionine free medium. The proteins synthesized 2 h after stress are analyzed by 10% SDS-PAGE. (C) 0, (1) 6.25, (2) 12.5, (3) 25, (4) 50, (5) 100 μM.

(Chang *et al.*, 1989). Synthesis of cadmium induced *hsps* was not detected in CCG cells up to 150 μM for 1 h (data not shown).

DISCUSSION

We studied the kinetics of *hsp* induction under various heat treatments in a cultured fish cell line CCG and the localization of *hsp*s before and after heat shock. In order to facilitate the comparison of the dynamic states of *hsp* production after various heat treatments, all results of *hsp* synthesis during various heat treatments were measured with a densitometer. The relative optical intensities are summarized in Table 1.

As can be seen from Table 1, CCG cells were induced to synthesize four *hsp*s at 37-43°C: *hsp*87, *hsp*70, *hsp*33 and *hsp*27. When CCG cells were heated at 43°C for 15 min, only the *hsp*70 was detected, and all others were suppressed. During a prolonged heat shock at 40°C, all *hsp*s were synthesized continuously and were maintained at high levels before 6 h;

Table 1
Relative optical intensities of *hsp*s synthesis under different heating conditions[1]

	43°C				40°C					40°C						37°C						
	3′, 5′, 10′, 15′[2]				5′, 10′, 15′, 30′, 60′					40°C						37°C						
	31°C[3]				31°C					4-6	8-10	36-38	60-62	84-86	117-119	4-6	8-10	36-38	60-62	84-86	117-119
*Hsp*s	0-2 h				0-2 h					h						h						
87	.25	.25	.25	.00	.22	.24	.43	.40	.41	.60	.35	.21	.21	.14	.19	.27	.25	.24	.20	.17	.16
70	.43	.48	.59	.28	.20	.46	.64	.69	.70	.89	.42	.37	.41	.28	.30	.53	.11	.17	.17	.13	.14
33	.03	.24	.31	.00	.03	.15	.24	.31	.38	.40	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
27	.03	.03	.10	.00	.05	.17	.27	.28	.27	.07	.59	.24	.21	.23	.22	.00	.00	.00	.00	.00	.00

[1] These include: (1) acute heating at 43° or 40°C for 3, 5, 10, 15, 30 or 60 min followed by 2 h labelling at 31°C, (2) chronic heating and labelling at 40°C or thermal adaptation and labelling at 37°C for 4-6, 8-10, 36-38, 60-62, 84-86, 117-119 h.

[2] Heating condition.

[3] Labelling condition.

however, at 37°C, only high molecular weight *hsp*s were continuously synthesized, although even these were synthesized in reduced quantities. Similar results have been obtained from other fish cell lines (TO-2 and CCF), in which no enhancement of low molecular weight *hsp*s was observed during thermal adaptation (Chen *et al.*, 1988 and unpublished data). These results suggest that synthesis of low molecular weight *hsp*s in cells may not be required for growth during thermal adaptation. Moreover, in yeast, *Saccharomyces cerevisiae*, Petko and Lindquist (1986) used disruption and deletion mutation methods to show that *hsp*26 is not required for growth at high temperatures (Petko and Lindquist, 1986).

In this study, the synthesis of *hsp*27 was detected only when the rate of *hsp*33 synthesis declined. However, pulse-chasing and immunoblotting tests (unpublished data) indicated that there was no antigenic relation between them. In addition, the synthesis of *hsp*27 required increased stress to reach its maximal rate (Figs. 1, 2 and 4). The results of analyses of three color carp cell lines with two-dimensional gels (unpublished data) showed that the low molecular weight *hsp*s were different from pI values and, therefore, were considered to be tissue-specific.

When we compared the proteins induced by various heat treatments or arsenite treatments in CCG cells, we found that the most prominent polypeptide induced by all treatments was *hsp*70. Again, immunofluorescence experiments indicated that *hsp*70 was mainly located in the nucleus after heat shock. These results reinforce the hypothesis that *hsp*70 closely confers protection to cells against thermal damage.

In conclusion, the intensity of the response in CCG cells varies with both the magnitude of the heat shock and the duration of exposure to the stress. In other words, the stress response is not regulated in an all-or-none manner. The same result has been observed by many others (Arrigo *et al.*, 1988; Berger and Woodward, 1986; Chen *et al.*, 1988; Craig, 1985; Gedamu *et al.*, 1983; Henle and Dethlefsen, 1978; Kothary and Candido, 1982; Kothary *et al.*, 1984; Lindquist, 1986; Misra *et al.*, 1989; Mosser *et al.*, 1986; Schlesinger, 1986; Welch and Feramisco, 1984). Much work remains to be studied about the *hsp*s, and their physiological function in fish cells. For these purposes, our efforts, through the use of immunofluorescent assays, are aimed at examining the dynamic state and localization of *hsp*s in the color carp cells after heat shock.

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熱休克蛋白質在錦鯉魚鰓細胞內的表現

古鎮鈞　陳秀男

錦鯉魚鰓細胞 (CCG) 在高溫的環境下會有四種熱休克蛋白質，*hsp*87、*hsp*70、*hsp*33 和 *hsp*27 過量產生。分別研究它們在 37°，40° 和 43°C 受熱環境下的合成速率，發現在上述各受熱條件下 *hsp*70 的合成速率爲四種熱休克蛋白質中最快也最明顯的，而 *hsp*27 則最慢。當在 40°C 環境下持續受熱六小時後 *hsp*87 和 *hsp*70 的合成速率降低了，*hsp*33 更停止生產，但 *hsp*27 則仍舊繼續合成。然持續在 37°C 環境下受熱時則只有 *hsp*87 和 *hsp*70 兩熱休克蛋白質在合成。

以螢光免疫法探測 CCG 細胞在 40°C 受熱 10 小時後的分布位置時，發現 *hsp*70 在受熱後會主要出現在細胞核，而 *hsp*33 和 *hsp*27 則僅出現在細胞質，以及伴隨在細胞核四周的明顯顆粒體。當放回 31°C 24 小時後，*hsp*70 已完全消失，而 *hsp*33 和 *hsp*27 則仍以顆粒體表現於細胞質內。細胞先經 Actinomycin D 處理可抑制所有熱休克蛋白質產生，顯示熱休克蛋白質的產生受控於 DNA 的轉譯作用。除了 *hsp*70 外，*hsp*87、*hsp*33 和 *hsp*27 均會在電泳過程中自行分解 (self-degradation)，這點和果蠅或老鼠的細胞株只發生在 *hsp*70 的現象不一樣。